DOI: 10.1534/genetics.104.029223

Cyclin B-Cdk Activity Stimulates Meiotic Rereplication in Budding Yeast

Randy Strich,*,[1] Michael J. Mallory,* Michal Jarnik* and Katrina F. Cooper†

*Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111 and
†Department of Biochemistry, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102

Manuscript received March 23, 2004
Accepted for publication April 30, 2004

ABSTRACT

Haploidization of gametes during meiosis requires a single round of premeiotic DNA replication (meiS) followed by two successive nuclear divisions. This study demonstrates that ectopic activation of cyclin B/cyclin-dependent kinase in budding yeast recruits up to 30% of meiotic cells to execute one to three additional rounds of meiS. Rereplication occurs prior to the meiotic nuclear divisions, indicating that this process is different from the postmeiotic mitoses observed in other fungi. The cells with overreplicated DNA produced asci containing up to 20 spores that were viable and haploid and demonstrated Mendelian marker segregation. Genetic tests indicated that these cells executed the meiosis I reductional division and possessed a spindle checkpoint. Finally, interfering with normal synaptonemal complex formation or recombination increased the efficiency of rereplication. These studies indicate that the block to rereplication is very different in meiotic and mitotic cells and suggest a negative role for the recombination machinery in allowing rereplication. Moreover, the production of haploids, regardless of the genome content, suggests that the cell counts replication cycles, not chromosomes, in determining the number of nuclear divisions to execute.

MEIOSIS is the process by which diploid organisms produce haploid gametes capable of sexual reproduction. Following premeiotic DNA replication (meiS), the homologs synapse via a specialized protein structure termed the synaptonemal complex (SC; Zickler and Olson 1975), reviewed in Kupiec *et al.* (1997), Roeder (1997), and Zickler and Kleckner (1999). The proper execution of meiosis I, also called the reductional division, requires recombination for proper alignment at metaphase I (Klapholz and Esposito 1980b). The second meiotic or equational division follows meiosis I without an intervening S phase. Therefore, haploidization is achieved by performing one round of DNA synthesis followed by two successive nuclear divisions. In budding yeast, the four haploid genomes are encapsulated into individual spores that are contained within a single cell termed an ascus.

During meiosis, rereplication is prevented both at the normal meiS window and between meiosis I and meiosis II. The block to rereplication during mitotic cell division in budding yeast requires the destruction of the Cdc6p origin licensing factor, nuclear export of the Mcm2-7 helicase complex, and inactivation of the origin recognition complex (Nguyen *et al.* 2001). These pathways are activated by the cyclin B-Cdc28p kinase that promotes progression through G_2. Similarly, the block to replication between meiosis I and meiosis II in starfish or Xenopus oocytes requires Cdc6 destruction (Lemaitre *et al.* 2002; Whitmire *et al.* 2002), Mcm4 relocalization (Lindner *et al.* 2002), and high cyclin B-Cdk1 activity (Picard *et al.* 1996). Therefore, similar mechanisms appear to prevent unscheduled DNA replication during mitotic cell division and between meiosis I and meiosis II. The mechanism that blocks rereplication during the normal meiS window is still unknown.

Progression through the mitotic cell cycle is controlled by the temporal destruction of key regulatory proteins by ubiquitin and the 26S proteasome (reviewed in Hockstrasser 1995). The G_2-to-M transition during the mitotic cell cycle requires a ubiquitin ligase termed the anaphase promoting complex/cyclosome (APC/C; reviewed in Tyers and Jorgensen 2000). The activity and specificity of the APC/C is derived from the association by a member of the Cdc20 protein family (Sethi *et al.* 1991). Ama1p is a meiosis-specific member of the Cdc20 family that is required for transit through prophase I and the exit from meiosis II (Cooper *et al.* 2000). Consistent with its role at the end of meiosis II, Ama1p is required for the destruction of the major meiotic B-type cyclin Clb1p. In this report, we demonstrate that, opposite to mitotic cell division, ectopic activation B-cyclin activity is sufficient to stimulate additional rounds of DNA replication during the meiS window. The resulting spores are viable and haploid and demonstrate normal Mendelian inheritance, indicating that rereplication was complete and that the spindle apparatus was reprogrammed to accommodate the additional rounds of replication. Finally, rereplication is enhanced by preventing either normal synaptonemal com-

[1]*Corresponding author:* Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111. E-mail: randy.strich@fccc.edu

plex formation or recombination itself, suggesting a negative role for this process in modulating meiS reinitiation.

MATERIALS AND METHODS

Strains/plasmid constructions: The strains utilized in this study are derivatives of RSY335 (*MAT***a**/*MAT*α *cyh2R-z ho*::*LYS2 leu2*::hisG *lys2 trp1*::hisG *ura3-1*; Cooper *et al.* 1997) with the following exceptions. RSY883 (*MAT***a**/*MAT*α *ho*::*LYS2 /lys2 trp1*::hisG *ura3-1*) is a derivative of NKY278 (Alani *et al.* 1990) with *TRP1* deleted. RSY665 (*MAT***a**/*MAT*α *bar1 cdc16-1 gal1 leu2 trp1 ura3*) is a diplodized derivative of 2031-19A (a gift from V. Guacci, Fox Chase Cancer Center). All disruption alleles or epitope tags were constructed using PCR-based gene replacement (Longtine *et al.* 1998). The *ENO1-CLB1* (pKC-416) was constructed by inserting the *CLB1* coding region along with the *CYC1* terminator into the high-copy plasmid pJS21-C (Kaldis *et al.* 1998). pKC418 contains the same fusion gene inserted into pRS425 (Christianson *et al.* 1992). The *ENO1-CLB5* expression plasmid contains the *CLB5* coding region tagged with the hemagglutinin epitope (a gift from C. Wittenburg, Scripts Institute) placed downstream of the *ENO1* promoter in pJS21-C. The *CLB1* destruction box (Richardson *et al.* 1992) was mutated (RxxL to AxxA) using oligonucleotide-directed mutagenesis (Kunkel 1985). This derivative (*CLB1*$^{db\Delta}$) was maintained on a single-copy plasmid under the control of its own promoter. The functionality of Clb1p$^{db\Delta}$ was verified by complementation assays.

Meiotic time-course experiments/cell imaging protocols: The meiotic time-course experiments and fluorescence-activated cell sorter (FACS) analysis were conducted as described (Cooper *et al.* 1997; Cooper and Strich 2002). Spindle morphology was determined by indirect immunofluorescence as described (Pringle *et al.* 1991). Chromosome behavior was analyzed during meiosis using the insertion of tandem Tet operators in chromosome V and the Tet repressor-GFP fusion protein as described (Michaelis *et al.* 1997). Quantitation of meiosis I and II completion was obtained by direct counting of at least 200 DAPI-stained cells. Flow sorting was conducted using a Becton Dickinson FACStar^{PLUS} flow cytometer with FACS/DESK data collection software. High-resolution imaging of multads was performed as described previously (Cooper *et al.* 2000).

RESULTS

***CLB1* overexpression induces production of asci with more than four spores:** Mutants lacking *AMA1* fail to destroy the B-type cyclin Clb1p or to complete meiosis (Cooper *et al.* 2000). To determine whether continued expression of Clb1p affected meiotic progression, the *CLB1* gene was overexpressed using the strong constitutive *ENO1* promoter and a high-copy 2μ plasmid (pKC416; see MATERIALS AND METHODS). This plasmid was introduced into a wild-type diploid (RSY335) and the transformant was induced to enter meiosis. Ectopic *CLB1* expression did not reduce sporulation compared to the vector control (75 *vs.* 82%, respectively). Interestingly, 14% of the culture expressing *CLB1* contained asci with more than the usual four spores (arrows, Figure 1A). Ultrastructual analysis revealed an intact outer cell membrane extending around the spores, demonstrating that these asci represent a single cell (Figure 1B). For convenience, asci containing more than four spores will be referred to as multads in this report. The number of visible spores varied from 6 to 20/multad with 6–8 spored cells being the most commonly found (Figure 1C). In a synchronous meiotic culture, DAPI staining revealed that the appearance of cells containing more than four nuclei occurred immediately following the execution of meiosis II and reached a maximum shortly thereafter (Figure 1D). No cells containing more than four nuclei were observed in the vector control despite taking samples for 48 hr following the shift to sporulation medium.

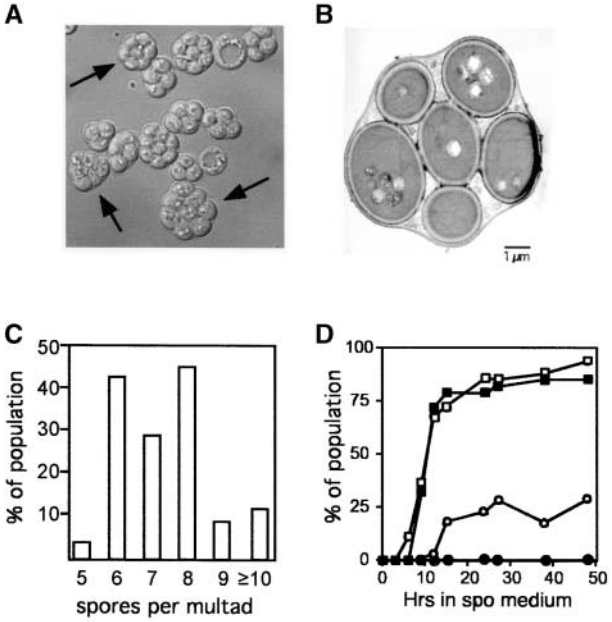


Figure 1.—Multad formation by ectopic *CLB1* expression. (A) Wild-type strain RSY335 harboring pKC416 was visualized by Nomarski optics following 24 hr in liquid sporulation medium. Arrows indicate asci containing more than four spores. Magnification, ×400. (B) Ultrastructure image of a typical multad cell. Final magnification, ×18,000. (C) The distribution of spore numbers found with a multad is presented as a percentage of the total counted (n = 56). (D) Meiotic time course was conducted with RSY335 harboring pKC416 (open symbols) or vector control (solid symbols). Cells were collected at the times indicated and the presence of bi- and tetranucleated cells (squares) or cells containing more than four nuclei (circles) were determined by DAPI staining.

Periodic transcription and protein degradation limit Clb1p accumulation from prophase I to the completion of meiosis II (Dahmann and Futcher 1995; Cooper *et al.* 2000). Conversely, the *ENO1-CLB1* gene maintained Clb1p at high levels throughout meiosis (data not shown). To determine whether the continued presence of Clb1p throughout meiosis was sufficient to induce multad formation, the destruction box motif (Glotzer *et al.* 1991; Richardson *et al.* 1992) was mutated, which protected the cyclin from ubiquitin-dependent degradation (data not shown). The destruction box mutant

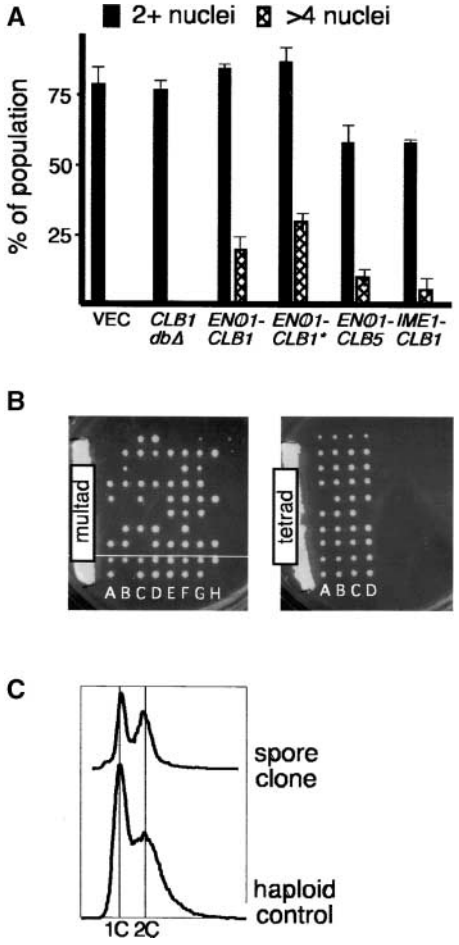


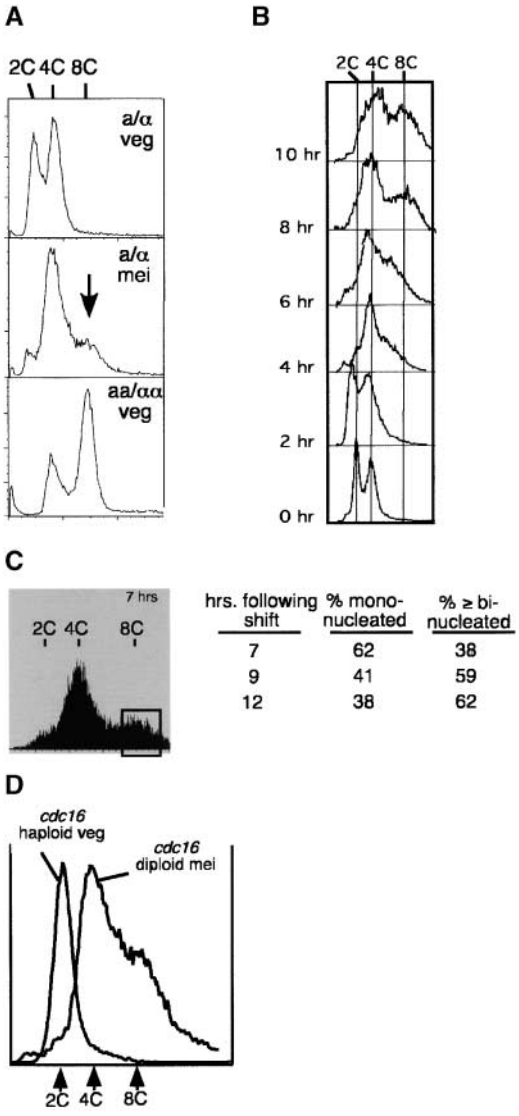
FIGURE 2.—Multad characterization. (A) RSY335 harboring the vector control, destruction box defective *CLB1* allele (*CLB1*$^{db\Delta}$) on single-copy plasmid, pKC416 (*ENO1-CLB1*), both pKC416 and pKC418 (*ENO1-CLB1**), pKC442 (*ENO1-CLB5*), or p2053 (*IME1-CLB1*) was sporulated in liquid culture. The percentages of the population containing two or more nuclei (solid bars) and more than four nuclei (hatched bars) are shown following 24 hr in sporulation medium. (B) Multads (left) or tetrads (right) were dissected onto rich medium and incubated at 30° for 2 days. (C) A representative multad spore clone selected from B was grown in rich medium to midlog phase and subjected to FACS analysis. A haploid control culture is included to indicate 1C and 2C DNA content.

allele (*CLB1*$^{db\Delta}$) was unable to induce multad formation (Figure 2A), indicating that high levels of Clb1p were a key requirement. Consistent with this possibility, the introduction of another plasmid harboring *ENO1-CLB1* resulted in a 70% increase over the single plasmid transformant. Multad formation was also observed when another B-cyclin (Clb5p) was overexpressed (Figure 2A). Finally, *CLB1* was placed under the control of the *IME1* early meiotic promoter (p2053; GUTTMANN-RAVIV *et al.* 2001) to determine whether restricting B-cyclin expression to meiosis was sufficient to induce multad formation. Indeed, a wild-type diploid harboring p2053 produced multads at levels similar to those of *ENO1-CLB5*. These results indicate that B-cyclin activity early in meiosis is sufficient to induce multad formation. In addition, these findings argue against any genomic alteration caused by overexpressing these cyclins during vegetative growth contributing to multad formation.

Spores derived from multads are viable and haploid: To characterize the spores derived from the multads, they were subjected to standard ascal microdissection and genetic analysis. Surprisingly, the viability of the spores (78%, averaged from three independent isolates) was only slightly reduced from the wild type (95%, Figure 2B). These findings indicate that each spore, in general, received a complete complement of chromosomes. The exact spore number did not influence spore viability as multads with 10 and 12 spores gave comparable viability (data not shown). Moreover, the resulting spore colonies demonstrated Mendelian marker segregation (*e.g.*, 4+:4−, 6+, 6−, etc.; data not shown), suggesting that normal nuclear divisions were occurring. To test whether these spores were haploid, two studies were performed. First, yeast cells can mate only if they are homozygous for the mating-type allele (*MAT***a** or *MAT*α). The spore colonies mated at 100% efficiency, indicating that only one mating-type locus was present in these cells. Finally, FACS analysis of four asynchronous spore cultures revealed the expected haploid DNA content (*e.g.*, Figure 2C). Although modest chromosome anomalies cannot be detected by these analyses, these studies suggest that multads completely rereplicate their genome one to three times and that chromosome segregation continues with high fidelity until a haploid genome is achieved.

Rereplication occurs during the normal meiS window: The genetic results indicate that the overexpression of B-type cyclins is able to induce rereplication of the genome in meiotic cells. This hypothesis was confirmed when an 8C peak was observed by FACS analysis of meiotic cells 10 hr following transfer to sporulation medium (Figure 3A). To examine the DNA replication kinetics during multad formation in more detail, a derivative of SK1 was transformed with pKC416 and pKC418. SK1 strains execute meiosis with higher synchrony and efficiency than do other backgrounds. FACS analysis of a meiotic time course with this transformant revealed that premeiotic S phase is complete by 4 hr (Figure 3B) with >4C cells evident only after 6 hr. The 8C cells were observed at 8 hr, 4 hr following the completion of meiS. Importantly, no >4C cells were detected in vegetative cultures (0 hr) or prior to premeiotic S phase, indicating that multad production was not the result of a polyploid strain as observed elsewhere (CID *et al.* 1998). In addition, the percentage of the population able to rereplicate their DNA increased significantly in the SK1 background (compare middle and top of Figure 3, A and B, respectively), suggesting that overall sporulation efficiency correlates well with multad production.

Other fungi exhibit postmeiotic mitoses producing asci with 8+ spores (SPRINGER 1993). In those examples,



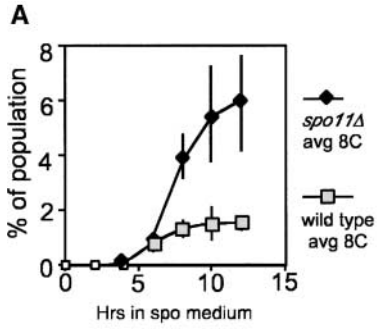
hrs. following shift	% mono-nucleated	% ≥ bi-nucleated
7	62	38
9	41	59
12	38	62

FIGURE 3.—Rereplication occurs prior to nuclear division. (A) FACS analysis of wild-type RSY335 harboring pKC416 either during mitotic cell division (veg) or following 10 hr in sporulation medium (mei). The tetraploid vegetative culture (bottom) indicates 4C and 8C genomic contents. (B) FACS analysis of RSY883 harboring pKC416 during meiosis at the time points indicated. The 2C, 4C, and 8C peaks are indicated. (C) Flow-sorted RSY883 8C cells were collected and the percentage of mononucleated and multinucleated cells in the population was determined (200 cells were counted). Mothers still attached to daughters and cell clumps were excluded from the count. (D) The RSY655 *cdc16-1* diploid transformed with pKC416 was induced to enter meiosis at the permissive temperature. Four hours following transfer to sporulation medium, the culture was shifted to restrictive temperature (34.5°) and samples were taken 8 hr later. The cells were fixed and subjected to FACS analysis. The arrested haploid *cdc16* parent of RSY655 was used as a 2C control (LAMB *et al.* 1994).

DNA replication occurs following the meiotic divisions. To determine when DNA rereplication occurs in cells overexpressing *CLB1*, two experiments were performed. First, flow sorting was used to isolate the 8C fraction from a sporulating culture (boxed population, Figure 3C). Fluorescent microscopic inspection of this sample revealed that the majority of the 8C cells were mononucleated at 7 hr, indicating that rereplication had occurred prior to nuclear division. As the time course continued, the fraction of multinucleated cells increased, as expected from earlier experiments (Figures 1C and 3B). Second, overreplication was observed in a *cdc16* mutant (Figure 3D) that arrests prior to meiosis I when shifted to the restrictive temperature (COOPER *et al.* 2000). Taken together, these experiments indicate that ectopic activation of B cyclin-Cdc28 recruits meiotic cells to rereplicate their DNA prior to nuclear division. These findings are consistent with previous studies in which B-type cyclin expression could substitute for the S-phase cyclins in meiotic cells (GUTTMANN-RAVIV *et al.* 2001; MOORE *et al.* 2003).

Rereplication is suppressed by the recombination pathway: In all organisms tested, meiS is two- to threefold longer than mitotic S phase (FORSBURG and HODSON 2000). The presence of Spo11p or Rec8p, two meiotic proteins required for initiating recombination and chromosome cohesion, respectively (KEENEY *et al.* 1997; KLEIN *et al.* 1999), has been shown to slow meiS completion (CHA *et al.* 2000), suggesting that the preparation of chromosomes for subsequent meiotic events slowed polymerase progression. We first tested the impact of Spo11p on rereplication. Two independent wild-type and two isogenic *spo11*Δ cultures transformed with the *ENO1-CLB1* expression plasmid were induced to enter meiosis. FACS analysis of samples taken during the time courses revealed that the initial appearance of the 8C peak was similar in both backgrounds (6 hr, Figure 4A). However, an approximate threefold increase was observed in the percentage of the *spo11* cultures able to produce 8C cells compared to wild type (6 ± 2 and 1.5 ± 0.1). These differences are unlikely to simply be due to the more rapid execution of DNA replication as observed previously for *spo11* mutants (CHA *et al.* 2000), as the percentage of 8C cells appears to have stabilized by 12 hr in both the wild-type and the *spo11* strains (Figure 4B).

Second, we examined multad formation in a mutant lacking *ZIP1*, a component of the SC (SYM *et al.* 1993). Two *ZIP1* wild-type and two *zip1* mutant cultures were transformed with pKC416. In most strain backgrounds (including the one used in these experiments), *zip1* mutants arrest meiotic progression in pachytene due to failed recombination (SAN-SEGUNDO and ROEDER 1999). However, the pachytene checkpoint is inactivated by Clb1p overexpression, allowing normal sporulation (LEU and ROEDER 1999; Figure 4B). The examination of these cultures 24 hr following transfer to sporulation medium revealed that the *zip1* mutants produced approximately threefold more multads than did the wild-type controls (Figure 4B, two independent experiments). Taken together, these results suggest that the preparation for,



B

	% multad		% total ≥2 nuclei	
	WT	*zip1Δ*	WT	*zip1Δ*
trial 1	9 ±1	26±14	75±6	76±13
trial 2	7±2	21±7	80±4	81±5

FIGURE 4.—The recombination pathway inhibits rereplication and multad production. (A) Two independent RSY335 and isogenic *spo11* mutants transformed with pKC416 were induced to enter meiosis. The average percentage of 8C cells as determined by FACS analysis is indicated. (B) Two wild-type and two *zip1* mutants (see MATERIALS AND METHODS) transformed with pKC416 were induced to enter meiosis and multad formation (more than four DAPI-staining nuclei) was determined 24 hr following transfer to sporulation medium. Two separate trials of independent transformants are presented. The total percentage of cells executing at least one meiotic division is shown on the right.

and execution of, recombination negatively impacts rereplication and subsequent multad formation.

The spindle checkpoint is active during multad nuclear divisions: The high viability of the multad spores indicated that chromosome segregation occurs with high fidelity. Proper chromosome disjunction requires the presence of a spindle checkpoint pathway to ensure proper alignment during metaphase. The hallmark of a spindle checkpoint is the protection from severe inviability following treatment with the microtubule-destabilizing agent benomyl (LI and MURRAY 1991; SHONN *et al.* 2000). Benomyl was added to the sporulation culture prior to the completion of the second meiotic division (as determined by DAPI staining) before the appearance of more than four nucleated cells (Figure 5A). As expected, benomyl reduced the kinetics of both tetrad and multad accumulation. Microdissection of multads derived from the benomyl or control cultures revealed that the treated spores were able to form colonies at ~70% the efficiency of the untreated control, suggesting that a spindle checkpoint monitors multad nuclear divisions. The presence of a functional spindle checkpoint suggested that the nuclear divisions were conducted in a coordinated manner. Indeed, tetranucleated and sexanucleated cells were observed with similar spindle morphology (Figure 5B), indicating that the chromosome divisions were coordinated within a cell.

Multad division requires recombination and reductional divisions: The production of normal haploid products following rereplication indicated that rounds of nuclear division in addition to meiosis I and meiosis II had to occur. To begin exploring the nature of these nuclear divisions, we asked whether recombination was required for the production of viable multad spores. Mutants unable to execute recombination (*e.g.*, *spo11*) missegregate their chromosomes at meiosis I, resulting in severe spore lethality (KLAPHOLZ *et al.* 1985). No viable spores were obtained in *spo11* multad spores (52 spores were examined), suggesting that multads undergo recombination and the reductional meiosis I division. To test this model, a *spo13* mutant was employed. In most strain backgrounds, *spo13* mutants skip meiosis I and execute the second meiotic division forming two diploid spores or dyads (KLAPHOLZ and ESPOSITO 1980a). FACS analysis revealed that rereplication did occur in the *spo13* mutant overexpressing *CLB1* but not in the vector control (data not shown) although in >95% of the population only a single nuclear division was observed. These findings indicate that multad production requires at least one reductional division (see DISCUSSION).

Multad nuclear divisions display normal pairing and assortment: During meiosis I, the replicated sister chromatids pair with their homologs but segregate as a single unit. We next determined whether rereplicated sisters associated only with each other (top, Figure 5C) or whether they were free to associate with either of the other rereplicated homologs as well (bottom). To address this question, we visualized one copy of chromosome V by integrating a tandem repeat of the tetracycline operator sequences and expressing a Tet repressor-GFP fusion protein (MICHAELIS *et al.* 1997). As expected from previous studies, only one GFP signal was detected in vegetative cells and during premeiotic S phase (data not shown). Following 8 hr in sporulation medium when overreplication is observed, cells containing multiple GFP signals within a single DAPI staining body were observed (arrows, Figure 5D). These results again support our conclusion that rereplication occurs before nuclear division. In addition, they suggest that rereplicated sisters are not constrained into a single focus within the nucleus. As the cells progressed through meiosis I, two GFP signals were observed in 17% of the binucleated cells ($n = 200$; Figure 5E). Since this culture produced multads at 27% efficiency, the calculated percentage of overreplicated dyads with two GFP signals was ~60%. This frequency is consistent with a model that rereplicated sisters are free to associate with each other or the other two homologs prior to the first division (both scenarios, Figure 5C). As expected, the strain harboring the vector control exhibited only one GFP signal in 96% of the binucleated cells counted ($n = 200$). Given that the Tet operator sequences are integrated only 7 cM from the chromosome V centromere

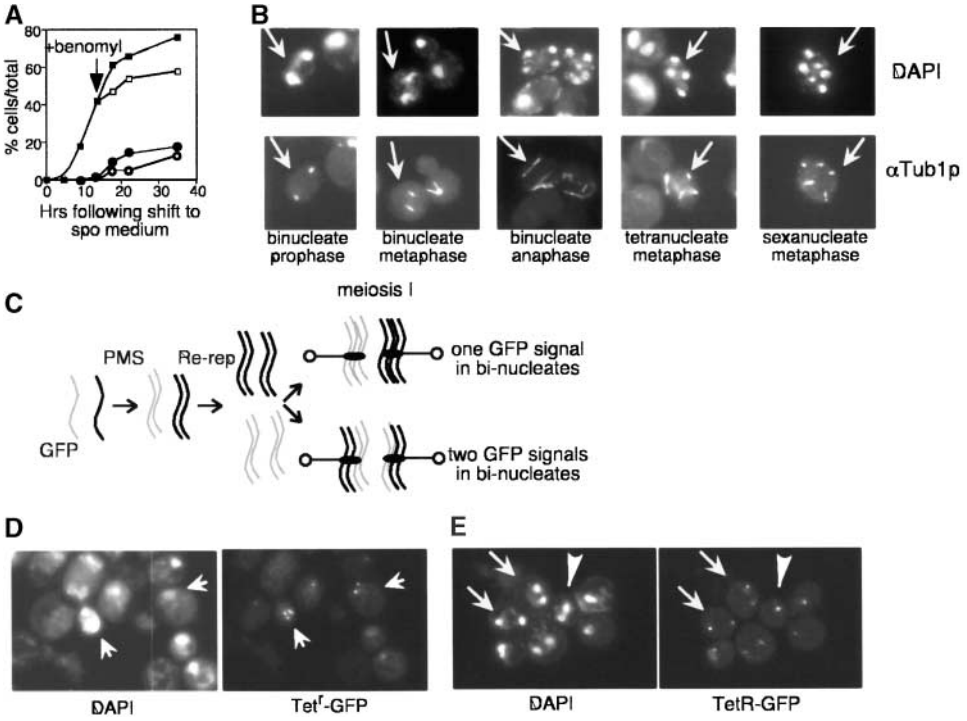


FIGURE 5.—Analysis of multad nuclear divisions. (A) Sporulating RSY335 harboring pKC416 was split at 12 hr with half receiving benomyl (60 μg/ml, open symbols) and the other half left untreated (solid symbols). The percentage of the culture that executed at least one meiotic division (squares) or possessed more than four nuclei (circles) was determined by DAPI staining. (B) The spindle status and nuclear morphology were determined by indirect immunofluorescence (αTub1p) and DAPI staining, respectively (COOPER and STRICH 2002). Arrows indicate cells containing the nuclear-spindle configuration listed under each panel set. (Left to right) The time points corresponding to each image pair were 10, 10, 12, 18, and 18 hr, respectively. (C) Model for pairing of rereplicated sister chromatids. The GFP-tagged chromosome V (shaded) can pair either with its other rereplicated sister (top) or with either of the rereplicated homologs (bottom). (D and E) The GFP-tagged chromosome V strain RSY726 (see MATERIALS AND METHODS) and nuclear morphology were followed during a meiotic time-course experiment. The arrows in D indicate mononucleated cells harboring multiple GFP signals. The arrows and arrowhead in E indicate cells containing a GFP signal in both or one nuclei following the first meiotic division, respectively. Magnification, ×400.

(*ura3*), it is unlikely that recombination between the centromere and Tet_{op} repeats could account for the high percentage of binucleated cells with a GFP signal in both nuclei. An alternative interpretation of these results is that overexpression of B-cyclins induces cohesion loss, thereby generating multiple GFP signals (GUACCI *et al.* 1994). However, the high viability of multad spores (Figure 2B) argues against this possibility. Taken together, our findings indicate that the segregation apparatus is flexible and able to accommodate the extra rounds of DNA synthesis. Moreover, the nuclear divisions appear remarkably normal in that they require recombination and demonstrate random assortment of replicated sister chromatids.

DISCUSSION

This report demonstrates that ectopic activation of cyclin B-Cdc28p kinase recruits meiotic cells to completely rereplicate their DNA. Many fungi possess programmed postmeiotic mitotic divisions (SPRINGER 1993). However, our finding that DNA rereplication occurs prior to nuclear division indicates that this process is not simply the activation of an analogous cryptic pathway in *Saccharomyces cerevisiae*. Meiotic rereplication is enhanced in strain backgrounds mutant for normal SC formation or recombination. These findings suggest that the extensive protein modification that occurs to prepare chromosomes for synapsis and recombination may provide at least a partial mechanism by which rereplication is blocked. Finally, the ability of rereplicated genomes to correctly segregate into haploid, viable spores indicates that the system regulating nuclear division is flexible and can be reprogrammed on the basis of the number of DNA replication cycles, not the overall ploidy of the cell.

The finding that the precocious activation of cyclin B-Cdc28p stimulates complete rereplication in meiotic cells is surprising for two reasons. First, meiotic induction in yeast requires exposure to medium that lacks nitrogen, phosphates, and a fermentable carbon source (reviewed in KUPIEC *et al.* 1997). This nutrient-deficient environment cannot therefore contribute substrates that are necessary for additional rounds of DNA replication. Rather, the cell's internal pools of nucleotides, as well as amino acids and lipids required for spore wall production, must supply these necessary building blocks. This requirement may explain why only a subset of the population can initiate another round of DNA replication. Second, the block to rereplication in mitotic cells is activated by the same B-cyclin-Cdk activity (NGUYEN *et al.* 2001) that stimulates rereplication in meiotic cells. These findings argue that the block to rereplication pathway is very different between meiosis and mitosis. The approximate two- to threefold increase in the 8C population or multad formation in *spo11* or *zip1* mutants suggests an alternative mechanism that may at least contribute to preventing rereplication. During

meiS, a protein structure is deposited on meiotic chromosomes required for synapsis, recombination, and reductional division (Klein *et al.* 1999; Watanabe and Nurse 1999; Borde *et al.* 2000; Murakami and Nurse 2001; Smith *et al.* 2001). One explanation is that rereplication is blocked during the meiS window by preparing the chromosomes for synapsis and recombination. If these protein structures are established concomitant with DNA synthesis, then reinitiation would be effectively blocked. Since this mechanism would not be in play during the interval between meiosis I and meiosis II, a system similar to that observed in mitotic cells may be installed, as observed in other systems (Picard *et al.* 1996; Lemaitre *et al.* 2002; Lindner *et al.* 2002; Whitmire *et al.* 2002).

One of the more surprising results from this study was that chromosome behavior appeared normal following rereplication cycles and that viable haploid progeny were produced. This finding is different from sporulating tetraploid cells that undergo one round of meiS and produce four diploid spores. Therefore, the cell is able to count replication cycles to reprogram the segregation machinery to execute the correct number of nuclear divisions. Currently, the mechanism by which the cell counts S phases is unknown. We demonstrate that recombination is necessary for multad spore viability, strongly suggesting that multad production requires an initial round of reductional division. Given that the execution of two sequential equational divisions leads to severe aneuploidy in *Schizosaccharomyces pombe rec8* mutants (Watanabe and Nurse 1999), we favor a model in which additional reductional divisions are performed until only replicated sister chromosomes remain to be segregated. A final equational division (meiosis II) triggers spore morphogenesis and the completion of the meiotic program. This model is consistent with our results that *spo13* mutants undergo only a single division that is most likely equational. In conclusion, this report demonstrates that the block to replication is different in meiotic and mitotic cells and that the segregation machinery is flexible and able to sort the additional chromosomes with high fidelity. Our finding that misregulation of only one gene is sufficient to dramatically alter the regulatory pathway governing meiotic DNA replication may shed light on the origin of some classes of germ cell malignancies (Carritt *et al.* 1982; Surti *et al.* 1990).

We thank E. Winter, A. Nicolas, V. Guacci, and T. Coleman for valuable comments on this article. We thank C. Wittenburg, K. Nasmyth, A. Nicolas, N. Kleckner, V. Guacci, and S. Keeny for strains and plasmids. This work was supported by grants from the National Institutes of Health (GM-57842) and the March of Dimes (FY01-240).

LITERATURE CITED

Alani, E., R. Padmore and N. Kleckner, 1990 Analysis of wild-type and rad50 mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:** 419–436.

Borde, V., A. S. Goldman and M. Lichten, 2000 Direct coupling between meiotic DNA replication and recombination initiation. Science **290:** 806–809.

Carritt, B., J. M. Parrington, H. M. Welch and S. Povey, 1982 Diverse origins of multiple ovarian teratomas in a single individual. Proc. Natl. Acad. Sci. USA **79:** 7400–7404.

Cha, R. S., B. M. Weiner, S. Keeney, J. Dekker and N. Kleckner, 2000 Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. Genes Dev. **14:** 493–503.

Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero and P. Hieter, 1992 Multifunctional yeast high-copy-number shuttle vectors. Gene **110:** 119–122.

Cid, V. J., R. Cenamor, M. Sanchez and C. Nombela, 1998 A mutation in the Rho1-GAP-encoding gene *BEM2* of *Saccharomyces cerevisiae* affects morphogeneisis and cell wall functionality. Microbiology **144:** 24–36.

Cooper, K. F., and R. Strich, 2002 Saccharomyces cerevisiae C-type cyclin *UME3/SRB11* is required for efficient induction and execution of meiotic development. Eukaryot. Cell **1:** 67–76.

Cooper, K. F., M. J. Mallory, J. S. Smith and R. Strich, 1997 Stress and developmental regulation of the yeast C-type cyclin *UME3* (*SRB11/SSN8*). EMBO J. **16:** 4665–4675.

Cooper, K. F., D. E. Egeland, M. J. Mallory, M. Jarnik and R. Strich, 2000 Ama1p is a meiosis-specific regulator of the anaphase promoting complex/cyclosome in yeast. Proc. Natl. Acad. Sci. USA **97:** 14548–14553.

Dahmann, C., and B. Futcher, 1995 Specialization of B-type cyclins for mitosis or meiosis in *S. cerevisiae*. Genetics **140:** 957–963.

Forsburg, S. L., and J. A. Hodson, 2000 Mitotic replication initiation proteins are not required for pre-meiotic S phase. Nat. Genet. **25:** 263–268.

Glotzer, M., A. W. Murray and M. W. Kirschner, 1991 Cyclin is degraded by the ubiquitin pathway. Nature **349:** 132–138.

Guacci, V., E. Hogan and D. Koshland, 1994 Chromosome condensation and sister chromatid pairing in budding yeast. J. Cell Biol. **125:** 517–530.

Guttmann-Raviv, N., E. Boger-Nadjar, I. Edri and Y. Kassir, 2001 Cdc28 and Ime2 possess redundant functions in promoting entry into premeiotic DNA replication in *Saccharomyces cerevisiae*. Genetics **159:** 1547–1558.

Hockstrasser, M., 1995 Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. Curr. Opin. Cell Biol. **7:** 215–223.

Kaldis, P., Z. W. Pitluk, I. A. Bany, D. A. Enke, M. Wagner *et al.*, 1998 Localization and regulation of the cdk-activating kinase (Cak1p) from budding yeast. J. Cell Sci. **111:** 3585–3596.

Keeney, S., C. N. Giroux and N. Kleckner, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384.

Klapholz, S., and R. E. Esposito, 1980a Isolation of *spo12-1* and *spo13-1* from a natural variant of yeast that undergoes a single meiotic division. Genetics **96:** 567–588.

Klapholz, S., and R. E. Esposito, 1980b Recombination and chromosome segregation during the single division meiosis in *spo12-1* and *spo13-1* diploids. Genetics **96:** 589–611.

Klapholz, S., C. S. Wadell and R. E. Esposito, 1985 The role of the *SPO11* gene in meiotic recombination in yeast. Genetics **110:** 187–216.

Klein, F., P. Mahr, M. Galova, S. B. Buonomo, C. Michaelis *et al.*, 1999 A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell **98:** 91–103.

Kunkel, T. A., 1985 Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:** 488–492.

Kupiec, M., B. Byers, R. E. Esposito and A. P. Mitchell, 1997 Meiosis and sporulation in Saccharomyces cerevisiae, pp. 889–1036 in *The Molecular and Cellular Biology of the Yeast Saccharomyces*, edited by J. R. Pringle, J. R. Broach and E. W. Jones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Lamb, J. R., W. A. Michaud, R. S. Sikorski and P. A. Hieter, 1994 Cdc16p, Cdc23p and Cdc27p form a complex essential for mitosis. EMBO J. **13:** 4321–4328.

Lemaitre, J. M., S. Bocquet and M. Mechali, 2002 Competence to replicate in the unfertilized egg is conferred by Cdc6 during meiotic maturation. Nature **419:** 718–722.

Leu, J. Y., and G. S. Roeder, 1999 The pachytene checkpoint in S. cerevisiae depends on Swe1-mediated phosphorylation of the cyclin-dependent kinase Cdc28. Mol. Cell **4:** 805–814.

Li, R., and A. W. Murray, 1991 Feedback control of mitosis in budding yeast. Cell **66:** 519–531.

Lindner, K., J. Gregan, S. Montgomery and S. E. Kearsey, 2002 Essential role of MCM proteins in premeiotic DNA replication. Mol. Biol. Cell **13:** 435–444.

Longtine, M. S., A. R. McKenzie, D. J. Demarini, N. G. Shah, A. Wach *et al.*, 1998 Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14:** 953–961.

Michaelis, C., R. Ciosk and K. Nasmyth, 1997 Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell **91:** 35–45.

Moore, J. D., J. A. Kirk and T. Hunt, 2003 Unmasking the S-phase-promoting potential of cyclin B1. Science **300:** 987–990.

Murakami, H., and P. Nurse, 2001 Regulation of premeiotic S phase and recombination-related double-strand DNA breaks during meiosis in fission yeast. Nat. Genet. **28:** 290–293.

Nguyen, V. Q., C. Co and J. J. Li, 2001 Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. Nature **411:** 1068–1073.

Picard, A., S. Galas, G. Peaucellier and M. Doree, 1996 Newly assembled cyclin B-cdc2 kinase is required to suppress DNA replication between meiosis I and meiosis II in starfish oocytes. EMBO J. **15:** 3590–3598.

Pringle, J. R., A. E. M. Adams, D. G. Drubin and B. K. Haarer, 1991 Immunofluoresence methods for yeast, pp. 565–610 in *Methods in Enzymology*, edited by C. Guthrie and G. R. Fink. Academic Press, San Diego/New York/London.

Richardson, H. E., D. J. Lew, M. Henze, K. Sugimoto and S. I. Reed, 1992 Cyclin B homologs in *Saccharomyces cerevisiae* function in S-phase and in G2. Genes Dev. **6:** 2021–2034.

Roeder, G. S., 1997 Meiotic chromosomes: it takes two to tango. Genes Dev. **11:** 2600–2621.

San-Segundo, P. A., and G. S. Roeder, 1999 Pch2 links chromatin silencing to meiotic checkpoint control. Cell **97:** 313–324.

Sethi, N., M. C. Monteagudo, D. Koshland, E. Hogan and D. J. Burke, 1991 The *CDC20* gene product of *Saccharomyces cerevisiae*, a β-transducin homolog, is required for a subset of microtubule-dependent cellular processes. Mol. Cell. Biol. **11:** 5592–5602.

Shonn, M. A., R. McCarroll and A. W. Murray, 2000 Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. Science **289:** 300–303.

Smith, K. N., A. Penkner, K. Ohta, F. Klein and A. Nicolas, 2001 B-type cyclins CLB5 and CLB6 control the initiation of recombination and synaptonemal complex formation in yeast meiosis. Curr. Biol. **11:** 88–97.

Springer, M. L., 1993 Genetic control of fungal differentiation: the three sporulation pathways of Neurospora crassa. BioEssays **15:** 365–374.

Surti, U., L. Hoffner, A. Chakravarti and R. E. Ferrell, 1990 Genetics and biology of human ovarian teratomas. I. Cytogenetic analysis and mechanism of origin. Am. J. Hum. Genet. **47:** 635–643.

Sym, M., J. A. Engebrecht and G. S. Roeder, 1993 ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell **72:** 365–378.

Tyers, M., and P. Jorgensen, 2000 Proteolysis and the cell cycle: with this RING I do thee destroy. Curr. Opin. Genet. Dev. **10:** 54–64.

Watanabe, Y., and P. Nurse, 1999 Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature **400:** 461–464.

Whitmire, E., B. Khan and M. Coue, 2002 Cdc6 synthesis regulates replication competence in Xenopus oocytes. Nature **419:** 722–725.

Zickler, D., and N. Kleckner, 1999 Meiotic chromosomes: integrating structure and function. Annu. Rev. Genet. **33:** 603–754.

Zickler, D., and L. W. Olson, 1975 The synaptonemal complex and the spindle plaque during meiosis in yeast. Chromosoma **50:** 1–23.

Communicating editor: A. P. Mitchell